

WHAT IS CLAIMED IS:

1. A method for identifying a protein which comprises the steps of:
 - (a) separating the protein from other proteins;
 - (b) digesting the protein, thereby forming first proteolytic peptides;
 - (c) acquiring the monoisotopic mass distribution spectrum of the first proteolytic peptides and acquiring the m/z values therefor;
 - (d) incorporating at least one amino acid 100% labeled with at least one stable isotope into the protein in a sequence-specific manner;
 - (e) separating the protein bearing the at least one labeled amino acid from other proteins;
 - (f) digesting the protein bearing the at least one labeled amino acid, thereby forming second proteolytic peptides;
 - (g) acquiring the monoisotopic mass distribution spectrum of the second proteolytic peptides and acquiring the m/z values therefor;
 - (h) comparing the monoisotopic mass distribution spectrum of the second proteolytic peptides with the monoisotopic mass distribution spectrum of the first proteolytic peptides to determine the amino acid composition of the first proteolytic peptides and the second proteolytic peptides, whereby the protein is identified from the m/z values of the first proteolytic peptides and the m/z values of the second proteolytic peptides and the amino acid composition of the first proteolytic peptides and the second proteolytic peptides.
2. The method as described in claim 1, wherein said step of incorporating the at least one 100% labeled amino acid into the protein in a sequence-specific manner further comprises the steps of:
 - (a) introducing the at least one 100% labeled amino acid into a cell capable of expressing the protein; and
 - (b) inducing the cell to express the protein.

3. The method as described in claim 2, wherein the cell is selected from a strain of cells containing genetic lesions for controlling amino acid biosynthesis such that endogenous amino acid biosynthesis and scrambling of the label to other types of residues are avoided.
4. The method as described in claim 3, wherein the strain of cells comprises *Escherichia coli*.
5. The method as described in claim 2, wherein said step of inducing the cell to express the protein is achieved using isopropylthiogalactoside.
6. The method as described in claim 2, wherein the at least one 100% labeled amino acid is selected from the group consisting of L-Methionine- d_3 and Glycine-2,2- d_2 .
7. The method as described in claim 1, wherein said step of digesting the protein, forming first proteolytic peptides and said step of digesting the protein bearing the at least one labeled amino acid, forming second proteolytic peptides are achieved using a protease.
8. The method as described in claim 7, wherein said protease comprises trypsin.
9. The method as described in claim 1, wherein said step of separating the protein and said step of separating the protein bearing the at least one labeled amino acid comprise the use of 2-D gels.
10. The method as described in claim 1, wherein said steps of separating the protein and said step of separating the protein bearing the at least one labeled amino acid comprise the use of liquid chromatography.
11. The method as described in claim 1, wherein said steps of obtaining the monoisotopic mass distribution spectrum of the first proteolytic peptides and acquiring the m/z values therefor, and obtaining the monoisotopic mass distribution spectrum of the second proteolytic peptides and acquiring the m/z values therefor are achieved using a mass spectrometer selected from the group consisting of matrix-assisted laser desorption/ionization time-of-flight mass spectrometers and electrospray mass spectrometers.

12. The method as described in claim 11, wherein postsource decay fragment ion spectra are acquired.
13. The method as described in claim 11, wherein delayed-extraction mass spectra are acquired.
14. The method as described in claim 2, wherein the at least one 100% labeled amino acid is diluted with variable amount of an unlabeled form thereof before said step of introducing the amino acid into a cell capable of expressing the protein, whereby the amino acid composition of each first peptide is confirmed.
15. The method as described in claim 1, wherein the at least one stable isotope is selected from the group consisting of ^{13}C , ^{15}N and ^2H .
16. The method as described in claim 1, wherein the monoisotopic mass distribution spectrum of a sufficient number of first proteolytic peptides and the m/z values therefor are acquired, and wherein the monoisotopic mass distribution spectrum of a sufficient number of second proteolytic peptides and the m/z values therefor are acquired such that the proteome is identified.
17. A method for identifying a protein which comprises the steps of:
 - (a) incorporating at least one amino acid 100% labeled with at least one stable isotope into the protein in a sequence-specific manner at a variable number of the sites for that at least one amino acid in the protein, forming thereby a mixture of partially labeled proteins;
 - (b) separating the mixture of partially labeled proteins from other proteins;
 - (c) digesting the mixture of partially labeled proteins, thereby forming proteolytic peptides; and
 - (d) acquiring the monoisotopic mass distribution spectrum of the proteolytic peptides and acquiring the m/z values therefor, whereby the protein is identified from the m/z values of the proteolytic peptides and the amino acid composition of the proteolytic peptides.
18. The method as described in claim 17, wherein said step of incorporating the at least one 100% labeled amino acid into the protein in a sequence-

specific manner at a variable number of sites for that at least one amino acid in the protein, further comprises the steps of:

- 5 (a) introducing the at least one 100% labeled amino acid and a chosen amount of an unlabeled same at least one amino acid into a cell capable of expressing the protein; and
- (b) inducing the cell to express the protein.
19. The method as described in claim 17, wherein the cell is selected from a strain of cells containing genetic lesions for controlling amino acid biosynthesis such that endogenous amino acid biosynthesis and scrambling of the label to other types of residues are avoided.
20. The method as described in claim 19, wherein the strain of cells comprises *Escherichia coli*.
21. The method as described in claim 18, wherein said step of inducing the cell to express the protein is achieved using isopropylthiogalactoside.
22. The method as described in claim 18, wherein the at least one 100% labeled amino acid is selected from the group consisting of L-Methionine- d_3 and Glycine-2,2- d_2 .
23. The method as described in claim 18, wherein the chosen amount of unlabeled at least one amino acid is 50%.
24. The method as described in claim 17, wherein said step of digesting the protein forming peptides is achieved using a protease.
25. The method as described in claim 24, wherein said protease comprises trypsin.
26. The method as described in claim 17, wherein said step of separating the mixture of partially labeled proteins comprises using 2-D gels.
27. The method as described in claim 17, wherein said steps of separating the mixture of partially labeled proteins comprises using liquid chromatography.

- 5
28. The method as described in claim 17, wherein said step of obtaining the monoisotopic mass distribution spectrum of the peptides and acquiring the m/z values therefor is accomplished using a mass spectrometer selected from the group consisting of matrix-assisted laser desorption/ionization time-of-flight mass spectrometers and electrospray mass spectrometers.
 29. The method as described in claim 28, wherein postsource decay fragment ion spectra are acquired.
 30. The method as described in claim 28, wherein delayed-extraction mass spectra are acquired.
 31. The method as described in claim 18, wherein the chosen amount of the unlabeled form of at least one amino acid is varied such that the amino acid composition of each peptide is confirmed.
 32. The method as described in claim 17, wherein the at least one stable isotope is selected from the group consisting of ^{13}C , ^{15}N and ^2H .
 33. The method as described in claim 17, wherein the monoisotopic mass distribution spectrum of a sufficient number of proteolytic peptides and the m/z values therefor are acquired such that the proteome is identified.

TABLE

**Theoretical Masses and Sequences of Proteolytic Fragments (>500 Da)
from Trypsin Digested UBL1**

Fragment#	Sequential#	Monoisotopic [M+]	Sequence
3*	18 -23	738.37	EGEYIK
7*	40 -45	730.39	MTTHLK
10	49 -54	785.33	ESYCQR
12	64 -70	896.46	FLFEGQR
		898.46	FLFEG (d ₂) QR
13	71 -78	895.46	IADNHTPK
11	55 -63	1001.52	QGVPMNSLR
		1003.52	QG (d ₂) VPMNSLR
		1004.52	QGVPM (d ₃) NSLR
5	26 -37	1359.69	VIGQDSSEIHK
1*	1 -16	1750.78	MSDQEAKPSTEDLGDK
14	79 -107	3643.63	ELGMEEEDVIEVYQEQT- GGHSTVLEHHHHHH
		3649.63	ELG (d ₂) MEEEDVIEVYQEQT- G (d ₂) G (d ₂) HSTVLEHHHHHH
		3646.63	ELGM (d ₃) EEEDVIEVYQEQT- GGHSTVLEHHHHHH

* - weak or missing peaks in MS peptide map.

Amino acid sequence of UBL1:

MSDQEAKPST EDLGDKKEGE YIKLKVIGQD SSEIHKVKM TTHLKKLKES YCQRQGVPMN
SLR FLFEGQR IADNHTPKELGMEEEDVIEVYQEQTGGHSTVLEHHHHHH